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EVOLUTIONARY STUDIES ON CTENUCHID MOTHS OF THE GENUS *AMATA*: 2. TEMPORAL ISOLATION AND NATURAL HYBRIDIZATION IN SYMPATRIC POPULATIONS OF *AMATA PHEGEA* AND *A. RAGAZZII*

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ABSTRACT. *Amata phegea* and *A. ragazzii* are two sibling species which occur sympatrically in several areas of Central and Southern Italy. The occurrence of certain enzyme loci, electrophoretically diagnostic (PGM, HK, EST-5), allowed the correct identification of all individuals, including hybrids. Population studies in the field carried out in sympatric areas of Central Italy revealed the occurrence of seasonal displacement in the flight period acting as a premating isolating mechanism between the two species, in spite of a partial overlapping. Premating behavior of homospecific and heterospecific pairs investigated in the laboratory showed the existence of isolating mechanisms also at the ethological level, which lowers the mating success of the heterospecific pairs. The frequency of hybrids in different localities varies from 0 to 0.053; the highest rates of hybridization were found in biotopes recently altered by man. Hybrid frequencies, similar in larval and adult stages, suggest a normal viability of the hybrids.

The ctenuchid moths *Amata phegea* (Linnaeus, 1758) and *A. ragazzii* (Turati, 1917) are two sibling species, so far scarcely investigated from the genetic, ecological, ethological and zoogeographical points of view. *A. phegea* is widely distributed in Central Western Europe (with the exception of the Iberian peninsula) and is particularly abundant and widespread in Italy; while *A. ragazzii* is endemic in Central Southern Italy. The latter species has to date been observed in Calabria, Campania, Lucania, Molise, Lazio and Umbria.

The two species presumably diverged through geographical isolation in warm refugia during the Pleistocene. They successively expanded their range in the post-glacial era following the spread of the deciduous broad-leaved forest. Areas of sympatric occurrence have been identified in the Alban Hills, in the Fioio Valley (Simbruini Mountains), near Leonessa (Rieti), at S. Polo dei Cavalieri (Sabine Mountains), at Mount Faito (Campania), at Viggiano (Lucanian Appennines) and in other areas of Central and Southern Italy.

At the morphological level identification of the adult of the two species may be difficult, particularly in females. On the other hand, allozyme separation by electrophoresis enables us to identify all individuals, both larvae and adults (Bullini et al., 1981). Using this

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method we were able to discover some hybrids in the overlapping areas although no evidence of introgression has been detected. This seems to indicate the existence of highly effective isolating mechanisms.

We are investigating these mechanisms both in the laboratory and in the field. In this paper we will present data showing patterns of temporal displacement in the flight period of the two species, probably representing an effective premating isolating mechanism; a further premating mechanism is also reported operating at the ethological level. In addition, we will present data on the occurrence of natural hybridization in some areas.

STUDY SITES, MATERIALS AND METHODS

As previously reported, a number of localities have now been identified in Central Italy where *A. phegea* and *A. ragazzii* occur sympatrically. In most of them the phenology of the two species was investigated by frequent observations over several years. More detailed information came from five localities near Rome, where periodic observations and counts were carried out.

The first locality is situated at a height of 850 m near Camerata Nuova, a village 50 km north east of Rome at the foot of the Simbruini Mountains. This site is characterized by a *Quercetum* association and represents the middle-lower part of the biotope described in detail by Sbordoni et al. (1979). The remaining four sites, namely Zagarolo, San Cesareo, Rocca Priora and Monte Cavo, were selected along an altitudinal transect 14 km long from 300 to 900 m above sea level within an area including the Alban Hills about 30 km south east of Rome. Most of this area is characterized by chestnut woods.

Observations were carried out from 1967 to 1980; reported data refer to 1974, which can be considered representative of the overall period. Direct counts of adult moths were employed to describe the phenology of *A. phegea* and *A. ragazzii*. Mark-release-recapture methods were also utilized to estimate absolute populations sizes of the two species (see Sbordoni et al., 1979).

Starch gel electrophoresis applied to three diagnostic enzyme loci: phosphoglucomutase (*Pgm*), hexokinase (*Hk*) and esterase (*Est-5*) was utilized to identify morphologically doubtful specimens and to discover hybrids (see Fig. 3). Electrophoretic techniques were, with minor modifications, those described by Ayala et al. (1972) for phosphoglucomutase and hexokinase, and by Selander et al. (1971) for esterase.

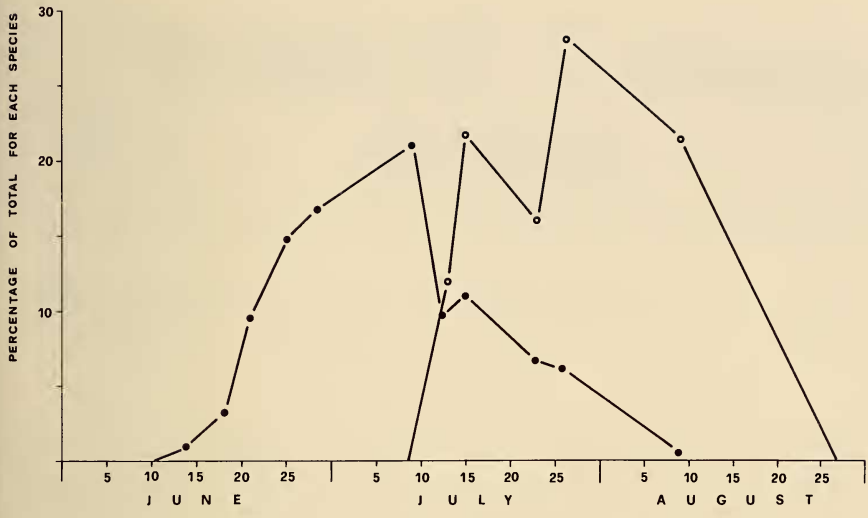

FIG. 1. Phenology of adult populations of *Amata phegea* (black circles) and *A. ragazzii* (white circles) at Camerata Nuova, Simbruini Mountains, 850 m, showing temporal displacement in the flight period.

RESULTS AND DISCUSSION

Temporal Displacement in Flight

By scattared observations and preliminary collection data we noted that the two species emerge at different times. This trend is clearly apparent from the data reported here.

At Camerata Nuova *Amata phegea* emerges about one month before *A. ragazzii* (Fig. 1). In the Alban Hills the emergence dates of the two species are separated by about 20 days, regardless of altitude (Fig. 2). Similar phenological data were observed in other localities in Central Italy. The degree of overlapping depends on the relative population sizes of the two species, which vary according to the localities. At Camerata Nuova *A. phegea* greatly outnumbers *A. ragazzii* and the ratio between the population sizes is approximately 30:1 (Sbordoni et al., 1979). More balanced situations were found in the localities of the Alban Hills. Here, consistent overlapping is limited to 10–15 days.

In both species males are first to emerge. Females are generally inseminated a few hours after their emergence (Stauder, 1927; Obraztsov, 1941). Female sex attractants are probably related to the anal papillae, which are rhythmically displayed by the virgin females (Obraztsov, 1966). Several males generally attempt to mate a single virgin female (Rasetti and Rasetti, 1921). Among adults the sex ratio is def-

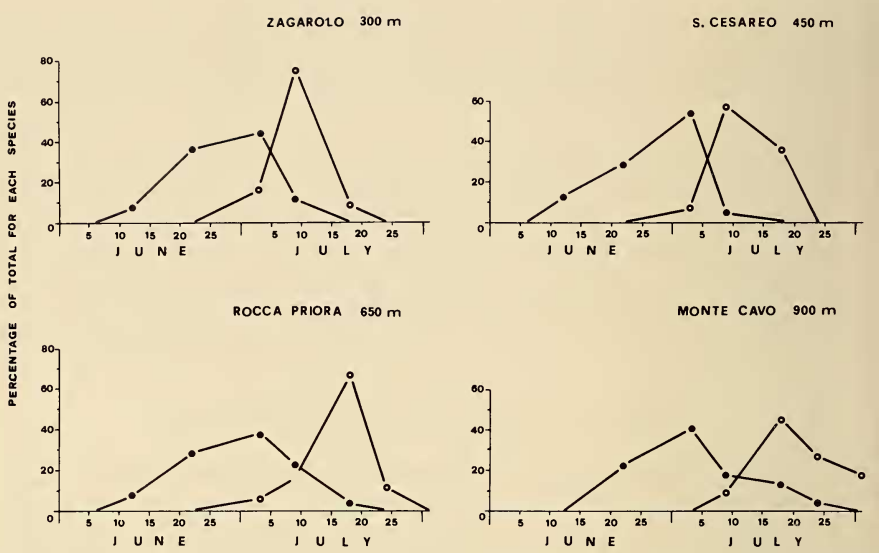

FIG. 2. Phenologies of adult populations of *Amata phegea* (black circles) and *A. ragazzii* (white circles) in 4 localities along an altitudinal transect south east Rome.

initely in favor of the males (80–85% in both species). According to these behavioral traits and the sex ratio, it seems improbable that a female of *A. phegea* could be inseminated by a male of *A. ragazzii*. On the other hand, old males of both species show an erratic behavior, and they tend to wander from their usual habitat in forest edges, open woods, etc. to open fields; also, this behavior limits the opportunities of mating between males of *A. phegea* and emerging females of *A. ragazzii*.

These observations led us to conclude that seasonal displacement may act as a premating isolating mechanism between *A. phegea* and *A. ragazzii*, although partial temporal overlapping occurs. This barrier would be particularly effective if monogamy were present in both species. However, this latter point needs to be tested.

Similar temporal displacement occurs also in other sympatric combinations of related *Amata* species. We observed this phenomenon in Istria and Dalmatia between *A. marjana* (Stauder, 1913) and *A. phegea*. In such instances, *A. marjana* is the first to fly, and the emergences of the two species are separated by about one month.

Premating Behavior

Besides the temporal displacement, an ethological isolating mechanism was also detected by preliminary laboratory experiments. *A.*

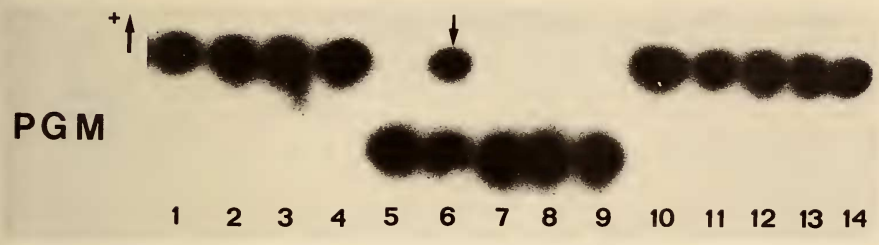

FIG. 3. Phosphoglucomutase (PGM) electrophoretic patterns of *Amata phegea*, *A. ragazzii* and their hybrid. *A. ragazzii* (specimens 1–4 and 10–14, from left to right) shows an anodic band migrating faster than *A. phegea* (specimens 5 and 7–9), whereas the hybrid (specimen 6) shows the two bands characteristic of the parental species.

phegea males isolated with conspecific virgin females are strongly attracted and approach the partner with a characteristic zig-zag flight. When the male touches the female she stops flying and the male lands on her. He repeatedly touches the costal area of her anterior wings with his antennae; the female, keeping her wings spread, raises her abdomen. Then copulation takes place. When *A. ragazzii* males are isolated with virgin *A. phegea* females, precopulatory behavior always begins later. Furthermore, the female often doesn't stop when touched and moves away. This generally interrupts a male's precopulatory behavior. Even when copulation takes place, the female frequently lays unfertilized eggs.

Natural Hybridization Between *A. phegea* and *A. ragazzii*

In this section we will present data demonstrating the occurrence of natural hybrids between the two *Amata* species occurring sympatrically in areas of Central Italy.

As reported in the materials and methods section, detection of hybrids by electrophoresis was made at some enzyme loci, which are diagnostic for *A. phegea* and *A. ragazzii* (*Pgm*, *Hk*, *Est-5*). At these loci no common allele is shared between the two *Amata* species studied. Hybrids are characterized by heterozygous patterns at each of these loci.

In Fig. 3 an example of a zymogram is shown, illustrating the pattern of *A. phegea*, *A. ragazzii* and their hybrid, at the *Pgm* locus.

Table 1 shows the numbers of *A. phegea*, *A. ragazzii* and hybrids in samples from some sympatric areas in Central Italy. Samples tabulated were collected during periods of temporal overlapping of the two species. However, if samples are collected over the whole period of flight of the two species, rates of hybridization may appear lower,

TABLE 1. Rates of natural hybridization between *A. phegea* and *A. ragazzii*, as revealed by electrophoresis on samples of adult moths from some sympatric areas of Central Italy.

Study site	Date of sampling	Number of specimens scored			Relative frequency of hybrids
		A. phegea	*A. ragazzii*	Hybrids	
Camerata Nuova	3–23 July 1974	173	21	1	0.005
Camerata Nuova	7 July–6 August 1975	891	99	3	0.003
Leonessa	25 July–8 August 1976	205	25	1	0.004
Montoro	25 June–23 July 1979	53	50	1	0.009
San Polo	4–19 July 1976	71	114	1	0.005
San Cesareo	8 July 1974	7	12	1	0.053
Tuscolo	8 July 1974	1	11	—	—
Monte Compatri	16 June–9 July 1974	39	10	—	—
Monte Porzio Catone	3–30 July 1975	10	202	1	0.004
Monte Cavo	30 July 1975	40	58	2	0.02

because all the hybrids detected were in flight relatively later, together with *A. ragazzii*.

Table 2 shows the data obtained from samples of larvae collected in three sites of the Alban Hills.

A comparison between the two tables do not reveal substantial differences in the frequency of hybrids between adult and larval stages from the same locality, suggesting normal viability of hybrids.

The frequency of hybrids varies from 0 to 0.053, but several values are around 0.004. The high rate of hybridization detected at San Cesareo, both from larval and adult samples observed in two distinct generations, may be tentatively related to the man-made alteration of their biotope. At the collection sites a wide zone of chestnut wood was replaced by *Rubus* sp.

Cases of sympatric hybridization are often associated with habitat alteration (Woodroof, 1973). In the case of *A. phegea* and *A. ragazzii* habitat alteration could affect the rate of hybridization and even the occurrence of sympatry between the two species. This working hypothesis requires further investigation.

TABLE 2. Rates of natural hybridization between *A. phegea* and *A. ragazzii*, as revealed by electrophoresis on samples of larvae from some sympatric areas of Central Italy.

Study site	Date of sampling	Number of specimens scored			Relative frequency of hybrids
		A. phegea	*A. ragazzii*	Hybrids	
San Cesareo	February 1975	50	107	6	0.038
Tuscolo	27 January 1975	5	113	1	0.008
Monte Compatri	February 1975	62	65	—	—

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